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ORIGINAL ARTICLE

Angiotensin-converting enzyme gene DD genotype neither increases susceptibility to acute pancreatitis nor influences disease severity

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Abstract

Background: The renin-angiotensin system (RAS) has been implied in the pathogenesis of various diseases including acute and chronic pancreatitis. Angiotensin-converting enzyme (ACE) is the key enzyme in activating the RAS. Deletion (D)-type polymorphism in the 16th intron of the ACE gene has been associated with higher serum levels of the enzyme. Inhibition of ACE was found to ameliorate acute pancreatitis in animal models suggesting that ACE plays a role in pathogenesis and progression of acute pancreatitis. Objectives were to investigate the occurrence of the ACE insertion/deletion (I/D) polymorphism in acute pancreatitis patients and its association with the severity of the disease.

Material and Methods: Seventy-nine acute pancreatitis patients and 95 healthy controls were evaluated. Acute pancreatitis cases were grouped as mild or severe according to the Atlanta criteria. Main outcome measure: The presence of the ACE I/D polymorphism.

Results: ACE gene I and D allele frequency of patients (44% and 56%) were similar to controls (45% and 55%, respectively). There were no significant differences in severity of pancreatitis between patients with the ACE-insertion or ACE-insertion/deletion versus ACE-deletion genotypes.

Conclusions: The ACE gene deletion polymorphism is neither a risk factor for development of acute pancreatitis nor contributes to the severity of disease or development of complications.

Keywords

acute pancreatitis, polymorphism, renin angiotensin system

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Introduction

Acute pancreatitis (AP) is a common acute inflammatory disease of the pancreas after injury through multiple aetiologies.[1] Pancreatic inflammation is initiated by pancreatic injury, most commonly through activation of trypsinogen and other pancreatic zymogens leading to autodigestion.[2] A number of environmental and genetic factors could be important in increasing susceptibility to acute pancreatitis. Once AP is initiated, the majority of patients have mild disease. However, about 20% progress to a severe course with increased mortality and morbidity.[3] The precise mechanisms determining the severity of the immune response and various complications in AP relative to the degree of injury are largely unknown. The identification of genetic risk factors would help to predict the risk and severity of the disease at an early stage.

Components of the renin-angiotensin system (RAS) contribute to the pathogenesis of various inflammation-associated diseases.[4] The RAS participates in the development of inflammation and fibrosis in the heart, kidney, lung and liver through the regulation of cell growth and inflammation, induction of oxidative stress and worsening of fibrosis.[5–7] The key enzyme in the RAS system is an angiotensin-converting enzyme (ACE) that converts angiotensin-I to the potent vasoconstrictor angiotensin-II.[8,9]

The RAS that is intrinsic to the pancreas plays a physiologic role in the regulation of pancreatic endocrine and exocrine

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functions.[10–12] Moreover, pathological conditions including acute pancreatitis and chronic hypoxia result in upregulation of the pancreatic RAS components.[13] Several studies have shown that inhibition of RAS may ameliorate the severity of experimental acute pancreatitis suggesting that variations in expression of ACE could play a role in the development or progression of acute pancreatitis.

The ACE gene insertion/deletion (I/D) polymorphism was discovered and defined within the last decade.[14] The deletion polymorphism of a 287-bp fragment of intron 16 of the ACE gene has been shown to result in higher levels of circulating enzyme in a dose-dependent manner.[14,15] Although the ACE DD genotype has been linked to several inflammatory diseases, the prevalence of the ACE polymorphism in subjects with acute pancreatitis versus controls and mild AP cases versus severe AP has not been reported. Recently, we reported that the ACE I/D polymorphism was not a risk factor in chronic pancreatitis.[16] This brought the hypothesis that the pancreas has its own local RAS and alternative enzymes that bypass ACE. We tested this hypothesis by investigating the prevalence of the ACE polymorphism in patients with acute pancreatitis compared with controls.

Materials and methods

Patients

The study was carried out with the approval of the institutional review board of the University of Pittsburgh Medical Center. Seventy-nine consecutive patients (41 males and 38 females, mean age 51.50 ± 19.71 years) admitted to the University of Pittsburgh Medical Center with acute pancreatitis were recruited through the pilot phase of the Severity of Acute Pancreatitis 1 (SAPS1) study. Written informed consent was obtained from all subjects and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki. Ninety-five healthy volunteers (40 male, 55 female, mean age 67.06 ± 9.96 years) recruited through the North America Pancreatitis 2 study were used as the control group.

The diagnosis of AP was based on the presence of abdominal pain and elevation of amylase and/or lipase serum levels at least three times above the upper limit of normal. The time interval between the onset of symptoms and admission to the hospital was less than 4 h. Acute Physiology and Chronic Health Evaluation (APACHE-II) score[17] and Ranson criteria scores[18] were calculated for all patients. Patients were classified into two groups according to APACHE-II and Ranson scores: mild AP cases who had peak APACHE-II score <8 or 48-h Ranson score <3 or severe AP if APACHE-II score was equal or greater than 8 and Ranson score was 3 or more.[19] The patients were only classified as severe AP cases if both criteria indicated so.

Laboratory procedure

Genomic DNA was purified from peripheral blood cells using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA).[20] The blood samples from many of the AP subjects were visibly abnormal, resistant to DNA separation with Puregene and necessitated an alternative DNA extraction process. DNA was purified using the QIA amp DNA blood mini kit (Qiagen, Valencia, CA, USA) as described by Scherczinger *et al.*[21] To determine the ACE genotype, a genomic DNA fragment on intron 16 of the ACE gene was amplified by PCR according to Rigat's method.[14] The amplified ACE gene fragments without insertion (D allele) and with insertion (I allele) of approximate 190 and 490 bp, respectively, were detected on 1% agarose gel containing ethidium bromide. To increase the specificity of DD genotyping, PCR amplifications were performed with an insertion-specific primer pair (5 prime TGGGACCACAGCGCCCGCCACTAC 3 prime and 5 prime TCGCCAGCCCTCCCATGCCCATAA 3 prime), that only the I allele produces a 335-bp amplicon. The 335-bp fragment was identified on 1.5% agarose gel containing ethidium bromide. The reaction yielded no products in the samples of DD genotype.[22]

Statistics analyses

Results are given as mean ± SD or allele frequencies. Armitage's trend test, standard odds ratio analyses and the one- and two-way analysis of variance (ANOVA) were used to compare AP cases and control subjects. 95% confidence interval and two-tailed *P*-values were calculated. The study was powered to detect a relative risk of three in AP cases (94.4%) (http://calculators.stat.ucla.edu/powercalc).

Results

The characteristics of the 79 AP patients (51.50 ± 19.71, male/female 41/38) are summarized in Table 1. The mean age of the healthy controls was significantly higher than the AP group ($P < 0.001$), whereas there was no difference in gender distribution ($P = 0.197$). The aetiology of pancreatitis was diagnosed as biliary in 23 patients, idiopathic in 19 patients and post-endoscopic retrograde cholangiopancreatography procedure (ERCP) in 20 patients, alcohol in 8 patients and other causes in the remaining. Median APACHE-II and Ranson disease severity scores were 5 (0–25) and 1 (0–8), respectively, in patients.

Sixteen patients were diagnosed as severe AP (mean age 59.25 ± 17.15 years; vs. mild cases $P = 0.078$ and vs. control group $P < 0.001$). The mean APACHE score was 14.5 (3–25) and Ranson score was 4 (1–8) in the severe AP group. Signs of remote organ failure including shock, pulmonary insufficiency and renal failure were present in all 16 subjects. Sixty-six patients (mean age 49.53 ± 19.96 years) were diagnosed as mild AP and had a median APACHE-II score of 4 (0–14) (vs. severe AP $P < 0.001$) and Ranson score of 1 (0–3) (vs. severe AP $P < 0.001$).

The frequency of ACE I/D genotypes are summarized in Table 2. Allele frequency of the ACE deletion was 54% in AP patients and 55% in healthy controls ($P = 0.452$). Frequency of the ACE-DD genotype compared with the II or ID genotype was not different between the AP group and controls ($P = 0.41$). Although the ACE-DD genotype was slightly higher in severe cases there was

Table 1 The demographic and clinical characteristics of acute pancreatitis patients

	Severe AP	Mild AP	Total	*P*-value
Age	59.2.1 ± 17.1	49.5 ± 19.9	51.50 ± 19.71	$P < 0.001$
Gender(male/female)	11/5	30/33	41/38	NS
Aetiology				NS
Biliary	5	18	23	
Alcohol	2	6	8	
Post-ERCP	4	16	20	
Hypertriglyceridemia	1	3	4	
Drugs	1	2	3	
Tumours	–	2	2	
Idiopathic	3	16	19	
Severity				
APACHE II	14.5	4	5	$P < 0.001$
(median, range)	(3–25)	(0–14)	(0–25)	
Ranson score	4	1	1	$P < 0.001$
(median, range)	(1–8)	(0–3)	(0–8)	

AP, acute pancreatitis; NS, non-significant; ERCP, endoscopic retrograde cholangiopancreatography procedure; APACHE, Acute Physiology and Chronic Health Evaluation.

Table 2 Genotype distribution of ACE gene in severe and mild acute pancreatitis and healthy controls

	Severe acute pancreatitis (*n* = 16)	Mild acute pancreatitis (*n* = 63)	Healthy controls (*n* = 95)	*P*-value
I (Allele frequency)	45%	45%	44%	NS
D (Allele frequency)	55%	55%	56%	NS
ACE-II	4 (25%)	14 (22%)	18 (19%)	NS
ACE-ID	6 (37.5%)	28 (45%)	50 (53%)	NS
ACE-DD	6 (37.5%)	21 (33%)	27 (28%)	NS

ACE, angiotensin-converting enzyme; NS, non-significant.

no significant difference between mild AP, severe AP cases and controls in genotype distribution ($P = 0.766$, Table 2).

When all patients were categorized according to their ACE genotype, the median APACHE-II score was 5 (0–25), Ranson score was 1 (0–8) and mean age 47.11 ± 17.25 years in patients with the ACE-DD genotype. Patients who carry the ACE-II or ACE-ID genotype had an APACHE-II of 5 (0–25), Ranson score of 1 (0–6) and mean age of 53.78 ± 20.67 years. Although patients with the ACE-DD genotype had slightly higher APACHE-II and Ranson scores the difference was not significant ($P = 0.393$, $P = 0.299$ and $P = 0.155$, respectively). There was no differences between patients with ACE-II, ID and DD genotypes in APACHE-II and Ranson scores whereas mean age was lower in patients with the ACE-DD genotype ($P = 0.135$, $P = 0.414$ and $P = 0.007$, respectively). There was no significant association between systemic complications of pancreatitis and the ACE-DD genotype.

Discussion

In the past decade, the association between genetic functional polymorphisms and inflammatory disease has drawn a great deal of attention. The majority of pancreatic diseases are associated with genetic polymorphisms.[23] Specific variations in the genomic DNA sequences of individuals strongly influence their susceptibility to pancreatitis, the nature and severity of the inflammatory process and the likelihood of complications.

Acute pancreatitis is a multifactorial inflammatory disease caused by the activation of several proteolytic enzymes and peptides.[24] The RAS system has been shown to play an important role in the regulation of pancreatic functions under pathological and physiological conditions.[12] RAS components may be the other mediators involved in the pathogenesis of acute pancreatitis. Angiotensin II, for example, stimulates the synthesis and secretion of vascular permeability factors,[25,26] adhesion molecules and chemokines.[27] It also induces migration and adhesion of monocytes and leukocytes.[28] Furthermore, tissue injury and acute inflammations induce production of circulating angiotensinogens and local angiotensins.[29] Indeed, Greenstein *et al.*[30] reported an increased plasma RAS activity in patients with AP. In animal models of AP and chronic pancreatitis with hypoxia, the components of local pancreatic RAS were markedly elevated.[11,31,32] Several studies demonstrated that inhibition of RAS attenuates

pancreatic inflammation in experimental model of acute pancreatitis.[33,34] These data indicate that changes in local RAS components could have a potential role in acute pancreatitis.

Based on the above observations, we hypothesized that functional polymorphisms in the ACE gene would alter the clinical course of AP. However, the ACE gene I/D polymorphism was not associated with susceptibility to AP or severity of AP. The ACE gene I/D allele frequencies were similar to previously reported frequencies in American studies[35] suggesting that the sample was representative of the larger population. On the other hand, it has been noted that the pathological risk of ACE-DD genotypes varies between populations with different genetic and environmental backgrounds,[36,37] so that the possibility of an important effect of this polymorphism in a polygenic or complex context cannot be excluded. The study may also have been underpowered as there was a trend towards the ACE-DD genotype being more frequent in severe AP patients. However, the effect would likely be small and there was no association between systemic complications of AP and ACE genotype.

Studies in animals demonstrate that components of the RAS influence the severity of acute pancreatitis.[32] Although an ACE plays an important role in the RAS, the factors influencing activation of ACE and regulation of ACE activity within various tissues may differ.[38,39] Added to tissue-specific factors are the possible roles of the activated pancreatic digestive enzymes, especially trypsin and chymotrypsin.[40] Chymotrypsin can act in a way similar to chymase to convert angiotensinogen to angiotensin I and II.[39] Likewise trypsin can act on components of the RAS in a way similar to tryptase to catalyse the conversion of angiotensin II into angiotensin III and IV. The balance of digestive enzyme activation and inhibition in the course of acute pancreatitis is probably complex, and not enough is known about local conditions to model this process. However, digestive enzyme activation may bypass ACE in regulating the RAS in the course of acute pancreatitis making the genotypic differences in ACE expression level or activity irrelevant.

The pancreas is a relative newcomer to the list of tissues with an intrinsic angiotensin-generating system. However, the present study shows that there is no relation between pathogenesis and progression of acute pancreatitis and the ACE I/D polymorphism.

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Conflicts of interest

None declared.

References

1. Isenmann R, Beger HG. (1999) Natural history of acute pancreatitis and the role of infection. *Baillie Res Best Pract Res Clin Gastroenterol* 13:291–301.
2. Bhatia M, Brady M, Shokuhi S, Christmas S, Neoptolemos JP, Slavin J. (2000) Inflammatory mediators in acute pancreatitis. *J Pathol* 190:117–125.
3. Neoptolemos JP, Raraty M, Finch M, Sutton R. (1998) Acute pancreatitis: the substantial human and financial costs. *Gut* 42:886–891.
4. Stroth U, Unger T. (1999) The renin-angiotensin system and its receptors. *J Cardiovasc Pharmacol* 33 (Suppl. 1):S21–S28; discussion S41–S43.
5. Ruiz-Ortega M, Ruperez M, Esteban V, Egido J. (2003) Molecular mechanisms of angiotensin II-induced vascular injury. *Curr Hypertens Rep* 5:73–79.
6. Bataller R, Gines P, Nicolas JM, Gorbig MN, Garcia-Ramallo E, Gasull X *et al.* (2000) Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 118:1149–1156.
7. Marshall RP, McAnulty RJ, Laurent GJ. (2000) Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor. *Am J Respir Crit Care Med* 161:1999–2004.
8. Pueyo ME, N'Diaye N, Michel JB. (1996) Angiotensin II-elicited signal transduction via AT1 receptors in endothelial cells. *Br J Pharmacol* 118:79–84.
9. Mii S, Ware JA, Mallette SA, Kent KC. (1994) Effect of angiotensin II on human vascular smooth muscle cell growth. *J Surg Res* 57:174–178.
10. Leung PS, Chappell MC. (2003) A local pancreatic renin-angiotensin system: endocrine and exocrine roles. *Int J Biochem Cell Biol* 35:838–846.
11. Leung PS, Chan WP, Wong TP, Sernia C. (1999) Expression and localization of the renin-angiotensin system in the rat pancreas. *J Endocrinol* 160:13–19.
12. Leung PS, Carlsson PO. (2001) Tissue renin-angiotensin system: its expression, localization, regulation and potential role in the pancreas. *J Mol Endocrinol* 26:155–164.
13. Leung PS. (2001) Local renin-angiotensin system in the pancreas: the significance of changes by chronic hypoxia and acute pancreatitis. *Jop* 2:3–8.
14. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346.
15. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D *et al.* (1992) Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359:641–644.
16. Oruc N, Lamb J, Kutlu OC, Barmada MM, Money ME, Slivka A *et al.* (2004) The functional angiotensin converting enzyme gene I/D polymorphism does not alter susceptibility to chronic pancreatitis. *JOP* 10:457–463.
17. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. (1985) APACHE II: a severity of disease classification system. *Crit Care Med* 13:818–829.
18. Ranson JH, Rifkind KM, Roses DF, Fink SD, Eng K, Spencer FC. (1974) Prognostic signs and the role of operative management in acute pancreatitis. *Surg Gynecol Obstet* 139:69–81.
19. Bradley EL, 3rd. (1993) A clinically based classification system for acute pancreatitis. *Ann Chir* 47:537–541.
20. Pfutzer RH, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J *et al.* (2000) SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 119:615–623.

21. Scherczinger CA, Bourke MT, Ladd C, Lee HC. (1997) DNA extraction from liquid blood using QIAamp. *J Forensic Sci* 42:893–896.

22. Yoshida H, Mitarai T, Kawamura T, Kitajima T, Miyazaki Y, Nagasawa R *et al.* (1995) Role of the deletion of polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J Clin Invest* 96:2162–2169.

23. Whitcomb DC. (2001) Hereditary pancreatitis: a model for understanding the genetic basis of acute and chronic pancreatitis. *Pancreatology* 1:565–570.

24. Lembeck F, Griesbacher T. (1996) Pathophysiological and possible physiological roles of kinins in the pancreas. *Immunopharmacology* 33:336–338.

25. Pupilli C, Lasagni L, Romagnani P, Bellini F, Mannelli M, Misciglia N *et al.* (1999) Angiotensin II stimulates the synthesis and secretion of vascular permeability factor/vascular endothelial growth factor in human mesangial cells. *J Am Soc Nephrol* 10:245–255.

26. Williams B, Baker AQ, Gallacher B, Lodwick D (1995) Angiotensin II increases vascular permeability factor gene expression by human vascular smooth muscle cells. *Hypertension* 25:913–917.

27. Schieffer B, Luchtefeld M, Braun S, Hilfiker A, Hilfiker-Kleiner D, Drexler H. (2000) Role of NAD(P)H oxidase in angiotensin II-induced JAK/STAT signaling and cytokine induction. *Circ Res* 87:1195–1201.

28. Alvarez A, Sanz MJ. (2001) Reactive oxygen species mediate angiotensin II-induced leukocyte-endothelial cell interactions in vivo. *J Leukoc Biol* 70:199–206.

29. Sun Y, Zhang J, Zhang JQ, Weber KT. (2001) Renin expression at sites of repair in the infarcted rat heart. *J Mol Cell Cardiol* 33:995–1003.

30. Greenstein RJ, Krakoff LR, Felton K. (1987) Activation of the renin system in acute pancreatitis. *Am J Med* 82:401–404.

31. Chan WP, Fung ML, Nobiling R, Leung PS. (2000) Activation of local renin-angiotensin system by chronic hypoxia in rat pancreas. *Mol Cell Endocrinol* 160:107–114.

32. Lai PB. (2001) Local renin-angiotensin system in the pancreas: the significance in acute pancreatitis. *Jop* 2:13–15.

33. Tsang SW, Ip SP, Wong TP, Che CT, Leung PS. (2003) Differential effects of saralasin and ramiprilat, the inhibitors of renin-angiotensin system, on cerulein-induced acute pancreatitis. *Regul Pept* 111:47–53.

34. Tsang SW, Ip SP, Leung PS. (2004) Prophylactic and therapeutic treatments with AT 1 and AT 2 receptor antagonists and their effects on changes in the severity of pancreatitis. *Int J Biochem Cell Biol* 36:330–339.

35. Beohar N, Damaraju S, Prather A, Yu QT, Raizner A, Kleiman NS *et al.* (1995) Angiotensin-I converting enzyme genotype DD is a risk factor for coronary artery disease. *J Investig Med* 43:275–280.

36. Pietinalho A, Furuya K, Yamaguchi E, Kawakami Y, Selroos O. (1999) The angiotensin-converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients. *Eur Respir J* 13:723–726.

37. Baudin B. (2002) New aspects on angiotensin-converting enzyme: from gene to disease. *Clin Chem Lab Med* 40:256–265.

38. Leung PS. (2004) The peptide hormone angiotensin II: its new functions in tissues and organs. *Curr Protein Pept Sci* 5:267–273.

39. Waldeck K, Lindberg BF, Persson K, Andersson KE. (1997) Characterization of angiotensin II formation in human isolated bladder by selective inhibitors of ACE and human chymase: a functional and biochemical study. *Br J Pharmacol* 121:1081–1086.

40. Ohta T, Amaya K, Yi S, Kitagawa H, Kayahara M, Ninomiya I *et al.* (2003) Angiotensin converting enzyme-independent, local angiotensin II-generation in human pancreatic ductal cancer tissues. *Int J Oncol* 23:593–598.